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# Cations in Motion: QM/MM studies of the dynamic and electrostatic roles of H<sup>+</sup> and Mg<sup>2+</sup> ions in enzyme reactions

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## Abstract

Here we discuss current trends in the simulations of enzymatic reactions focusing on phosphate catalysis. The mechanistic details of the proton transfers coupled to the phosphate cleavage is one of the key challenges in QM/MM calculations of these and other enzyme catalysed reactions. The lack of experimental information offers both an opportunity for computations as well as often unresolved controversies. We discuss the example of small GTPases including the important human Ras protein. The high dimensionality and chemical complexity of these reactions demand carefully chosen computational techniques both in terms of the underlying quantum chemical theory and the sampling of the conformational ensemble. We also point out the important role of Mg<sup>2+</sup> ions, and recent advances in their transient involvement in the catalytic mechanisms.

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## 1. Introduction

Computational techniques enable us to investigate systems of chemical and biological interest to a level of detail unavailable for most experimental methods. Biomolecular complexes, including enzymes, are one of the most challenging subjects due to their complexity in structure and function. Describing both the quantum chemistry required for accurate description of reactions as well as the complex structural/dynamical aspects of the protein/nucleic acid environment, hybrid quantum/classical QM/MM methods have a unique, unprecedented capability to examine reaction mechanisms at sub-atomic details. An extensive amount of work has been done toward understanding various enzymatic systems such as lipoxygenases [1,2], cofactor-free oxidases [3,4], Kemp eliminase [5,6], serine hydroxymethyltransferase [7] and many others [8–13]. Here we focus on phosphate catalytic reactions, which contribute probably the most important catalytic reactions in living organisms [14–19].

One of the key questions concerns the position of protons during reactions. In many organic reactions proton transfer (PT) is naturally part of the mechanism. Enzymes have evolved efficient catalytic mechanisms not to eliminate such PT events, but instead to include them, often in a concerted fashion. The positions of the protons, however, are usually not observed experimentally even in local reactant or product minima, not to mention transition states (TSs) or intermediates. Using QM/MM methods, we can study PT events together with the electron density changes leading to bond cleavage and formation at not only the local minima, but along the reaction path, at the rate limiting TS structures.

## 2. Phosphate catalytic reaction mechanisms

Phosphate cleavage and transfer is often coupled to PT as well (Figure 1): the attacking nucleophile has to be deprotonated, whereas the leaving group needs protonation to complete the reaction. Enzymes evolved not to deprotonate the incoming nucleophile *a priori*, (e.g. the lytic water is bound

in its neutral form instead of a hydroxide anion in the reactant complexes), but the deprotonation takes place together with the nucleophilic substitution [20,21]. To facilitate such cooperativity, the  $pK_a$  of the participating groups at the enzyme active sites is tuned in an optimal way using charged residues and metal ions, particularly  $Mg^{2+}$ . This cooperativity therefore is sensitive to mutations resulting in diminishing activity [21]. These details of the mechanism can be extended to most enzymes and experimental evidence in numerous examples shows that proton transfer is part of the rate limiting step [22–25].

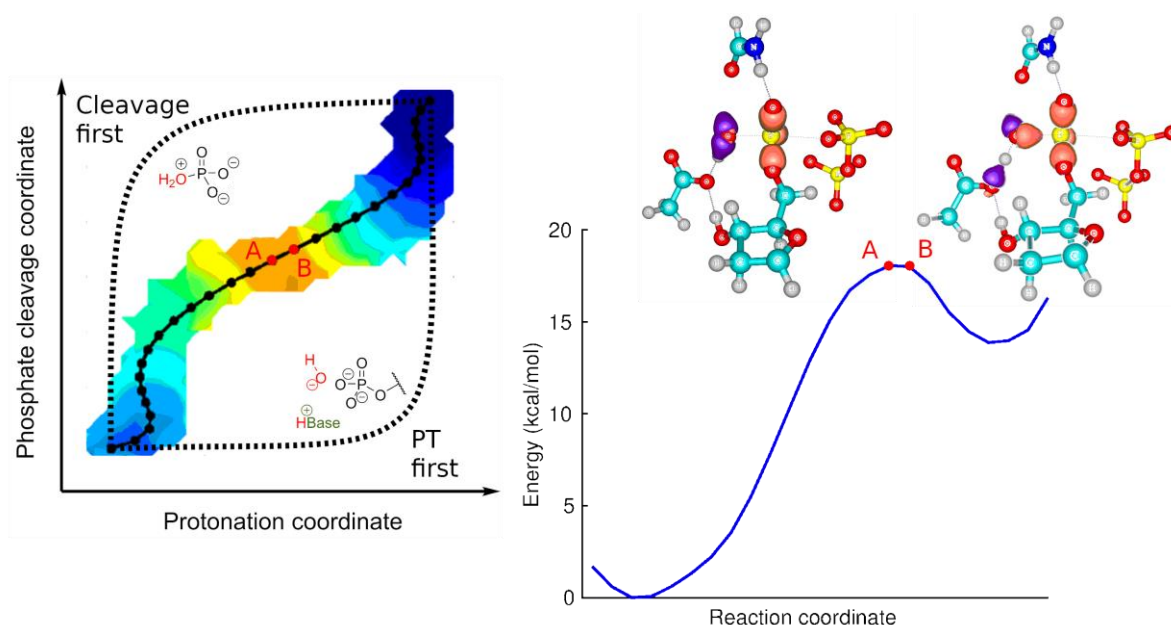


Figure 1. Illustration of coupled proton transfer events in phosphate hydrolysis. *Left*: dots and line represent a typical concerted path for (wild type) enzymes (example from ref [20]); dashed lines depict sub-optimal pathways: ‘PT first’ requires strongly basic conditions, while ‘cleavage first’ entail a more polarized phosphate. *Right*: Natural Bonding Orbitals (NBOs) before (A) and after (B) the coupled phosphate hydrolysis transition states in wild type dUTPase [21]. Once the new P-O bond is formed, the proton transfer to the carboxylate also took place.

### 3. Methodological Challenges

Describing the electronic structures of phosphate species requires accurate quantum chemical treatment. Most simple semiempirical methods fail to obtain the correct molecular structures in the active site, especially considering Mg-coordination geometries for the ligating phosphate groups. Density Functional Theory (DFT) is the sweet spot in terms of accuracy *versus* cost and therefore a frequently used QM method in these calculations. Alternative, more affordable methods use bespoke semiempirical algorithms such as DFTB [26–28] where standard semiempirical methods fail. In an effort to optimize accuracy and costs, mixed QM algorithms can also be used such as ONIOM [7,18,29]. Cluster QM calculations offer another approach, simplifying the problem by eliminating the MM degrees of freedom [30].

Exhaustive sampling of all conformational states is unavailable in larger systems using QM/MM calculations, therefore posing a key problem for determining accurate free energies. Works relying on minimizations from selected structures limit the conformational sampling available from the enzyme residues nearby the active site and a harmonic approximation to the free energy. When experimental structural information is not fully available, or inactive structures are used as the starting point, the sampling is key to bring the system into the catalytically competent conformation. Many studies that

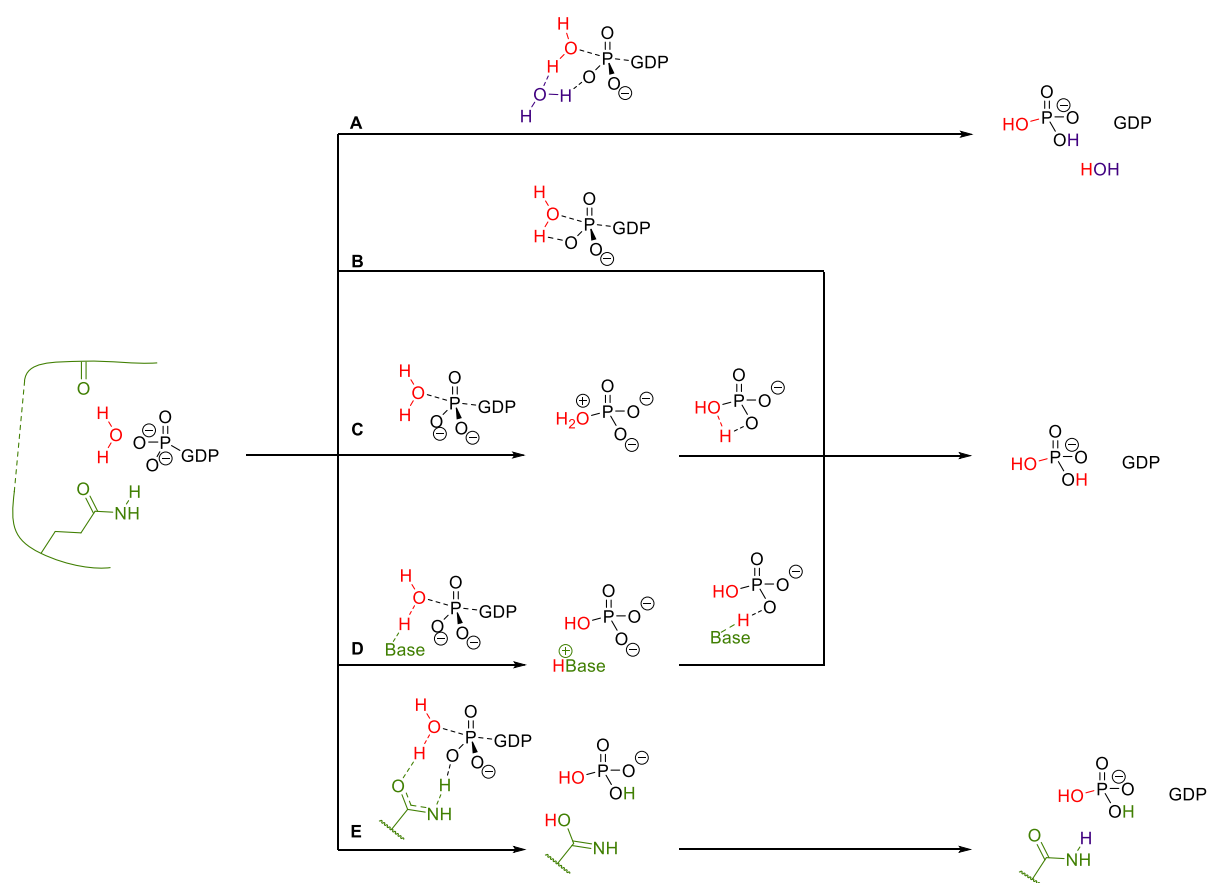
lack proper sampling have an incorrect/inactive structure as starting points, which may limit the conclusions reached [31,32]. This is particularly important in terms of the binding modes of metal ions.

Force field-based methods (*e.g.* EVB) are more cost-effective, enable longer simulation times, hence offer better sampling. However, they require parameterization for each possible intermediate, and can thus only probe specific pre-selected mechanisms, *e.g.*, novel PT events cannot take place automatically. Generally, there is no golden standard method for studying enzymatic mechanisms [33], as one needs to find a balance of effective sampling and computational cost/accuracy. Nevertheless, QM/MM is conceptually more appropriate to explore mechanistic possibilities, combined with an effective sampling algorithm, while other alternatives can still provide sufficient insight examining a selected mechanism.

Free energy calculations can help overcome conformational issues. Unbiased molecular dynamics (MD) simulations would require prohibitively long simulation times to escape from local minima, especially related to metal ion binding modes, large conformational changes and reaction barriers. Therefore, enhanced sampling simulations are required where bias is used along relevant collective variables. Umbrella sampling [34], metadynamics [35] and other adaptive biasing algorithms [36] are very efficient and often used successfully, particularly when a single reaction coordinate can be identified. However, in complex reaction mechanisms where several PT events need to be considered together with phosphate bond formation and breaking, these methods do not efficiently handle the high dimensional problem. Here, we and others developed string-type methods, which take the biasing problem to high dimensional space of collective variables in an efficient way [37–43]. Umbrella sampling type methods scale exponentially with the dimensionality of the collective variable, however, string methods define a one dimensional path that can be mapped onto high dimensional spaces efficiently. These paths are optimized iteratively, either using series of minimizations, which often have convergence problems, or, at finite temperatures, using molecular dynamics simulations in the string windows [38]. To further enhance sampling in orthogonal degrees of freedom, Hamiltonian replica exchange can be employed between the string images at virtually no added cost [40]. The obtained trajectories over all iterations can be unbiased using statistically optimal algorithms, such as the binless WHAM, to obtain reliable free energy landscapes [44–47].

#### **4. Phosphate Hydrolysis**

Despite the efforts to understand phosphate hydrolysis as an enzyme catalysed reaction, there are still several different reaction mechanisms promoted based on different computational methods/studies. Here we summarize potential mechanistic pathways for the gamma phosphate hydrolysis of nucleoside triphosphates as depicted in Figure 2. These differ in the key feature: how and when the PTs occur from the nucleophile water to the  $\gamma$ -phosphate with respect to the phosphate cleavage. Table 1 showcases that many studies obtain similar barriers to ones derived from kinetic experiments, although current methods are not expected to be more accurate than a few kcal/mol, heavily depending on the level of theory and sampling efficiency. Additionally, experimental measurements are also expected to have an error bar of 1-2 kcal/mol, not even considering changes due to the cellular environment.



**Figure 2.** Different mechanisms of phosphate hydrolysis shown on the example of GTP. Schematic transition states are depicted on the arrows. We define five different mechanistic pathways: **A**: two-water mechanism; **B**: one-step substrate assisted mechanism; **C**: two-step substrate assisted mechanism; **D**: base-catalysed mechanism; **E**: imide mediated mechanism. Calculated barriers for these variations are summarized in **Table 1** with various enzyme/substrate complexes.

**Table 1.** Recent computational results on triphosphate hydrolysis for several enzymes (wild type, WT, or mutation as specified) and/or their respective reactions in water. The corresponding mechanisms (**A-E**) are depicted in **Figure 2**. Experimental barriers are also shown (when available) as comparison with the computational work. Density functional is specified when applicable. Energetic data is given in kcal/mol.

Mechanism	Enzyme <sup>†</sup>	Mutation	Substrate	Calc. barrier	Exp. barrier	Method	Ref
<b>A</b>	EF-G <sup>a</sup>	WT	GTP	14.2	14.1	EVB	[48]
	EF-G <sup>a</sup>	H87A		24.7	>22.0		
	EF-Tu <sup>b</sup>	WT		13.8	<14.0		
	EF-Tu <sup>b</sup>	D21A		18.3	18.4		
	EF-Tu <sup>b</sup>	H84A		21.4	21.2		
	EF-Tu <sup>b</sup>	H84 <sup>NP</sup>		17.1			
	EF-Tu <sup>b</sup>	H84Q		18.5	~18.4		
	FeoB <sup>c</sup>	E66A E67A		18		DFT PBE/Amber metadynamics	[49]
	MeTP	-	MeTP	33		DFT BLYP	[50]
	Mg <sup>2+</sup>	-	AcP	15.3 (13.4)	23.9	DFT ω-B97X-D (M11L)	[51]
	Mg <sup>2+</sup>	-	MeTP	29.2 (22.6)			
	MMD <sup>d</sup>	WT	ATP	19	15-17	DFTB/CHARMM MFEP	[27]

	None	-	AcP	23.9 (21.9)	24.3	DFT $\omega$ -B97X-D (M11L)	[51]
	None	-	GTP	27	27	EVB	[48]
	None	-	MeTP	26.7 (33.0)	27.9	DFT $\omega$ -B97X-D (M11L)	[51]
	RasGAP <sup>e</sup>	WT	GTP	11		DFT PBE /CHARMM	[52,53]
<b>A*</b>	MMD <sup>d</sup>	WT	ATP	22	15-17	DFTB/CHARMM MFEP	[27]
<b>B</b>	Mg <sup>2+</sup>	-	AcP	31.4 (32.2)	23.9	DFT $\omega$ -B97X-D (M11L)	[51]
	Mg <sup>2+</sup>	-	MeTP	32.5 (23.9)	27.9		
	None	-	AcP	31.3 (31.2)	24.3		
	None	-	GTP	37.2		EVB	[54]
	None	-	MeTP	34.9 (35.6)	27.9	DFT $\omega$ -B97X-D (M11L)	[51]
	Ras <sup>f</sup>	WT	GTP	30.8		EVB	[54]
	RasGAP <sup>e</sup>	WT		28.5		DFT PBE/CHARMM	[52,53]
	RasGAP <sup>e</sup>	WT		28.7		EVB	[54]
<b>C</b>	EF-Tu <sup>b</sup>	WT	GTP	14.8		EVB	[48]
	FeoB <sup>c</sup>	WT		33		DFT PBE/Amber metadynamics	[49]
	None	-		29.7		EVB	[48]
	None	-		27.9			[54]
	Ras <sup>f</sup>	WT		23.9			
	RasGAP <sup>e</sup>	WT		14.9			
<b>D</b>	FeoB <sup>c</sup>	WT	GTP	17		DFT PBE/Amber metadynamics	[49]
	FeoB <sup>c</sup>	E66A		20			
	FeoB <sup>c</sup>	E67A		19			
	hGBP1 <sup>g</sup>	WT		18		DFT BLYP	[50]
	MMD <sup>d</sup>	WT	ATP	16	15-17	DFTB/CHARMM MFEP	[27]
	RasGAP <sup>e</sup>	Q61E	GTP	7		DFT PBE/CHARMM	[52,53]
<b>E</b>	RasGAP <sup>e</sup>	WT	GTP	14		DFT PBE/CHARMM	[52,53]

<sup>†</sup>None and Mg<sup>2+</sup> stands for simulation in bulk water in the absence or presence of Mg<sup>2+</sup>, respectively.

\*Involves a Ser sidechain.

<sup>a</sup>Elongation Factor G from *Thermus thermophilus* (4V90)

<sup>b</sup>Elongation Factor Tu from *Thermus thermophilus* (2XQD)

<sup>c</sup>Ferrous iron transport protein B from *Streptococcus thermophilus* (3SS8)

<sup>d</sup>Myosin II Motor Domain from *D. discoideum* (1VOM)

<sup>e</sup>Complex of human Ras and p120 GTPase activation protein (1WQ1)

<sup>f</sup>Human Ras (1QRA)

<sup>g</sup>Human guanine nucleotide binding protein-1 (2BC9 and 2B8W)

Path **A** is often termed as "two-water" mechanism as the proton transfer is facilitated by a second water molecule. It is likely to be the primary mechanism in bulk water [51]. This pathway was also found feasible in a number of studies [48,49], but it is not certain that the active site pocket can accommodate a second water, as these are not observed in crystallographic structures [53].

Pathways **B** and **C** both feature a PT via a four-membered ring, either in a concerted way, with the nucleophilic substitution on the phosphorus (**B**) or step-wise, via an intermediate (**C**). The strained ring structure does not provide an optimal arrangement for the basic moiety ( $\gamma$ -phosphate here) to donate electron density to the antibonding orbital of the breaking O-H bond. Thus, the activation barrier is likely to be underestimated by EVB or semi-empirical methods. In fact, similar barriers are obtained for both mechanisms **A** and **C** using EVB. Sometimes, these mechanisms are also called "substrate-assisted" as phosphate takes the proton directly from the water.

Path **D** covers all scenarios when the PT is facilitated by a Bronsted base, a residue that serves as the proton acceptor, resulting in a short-lived intermediate. This mechanism was found to be superior to

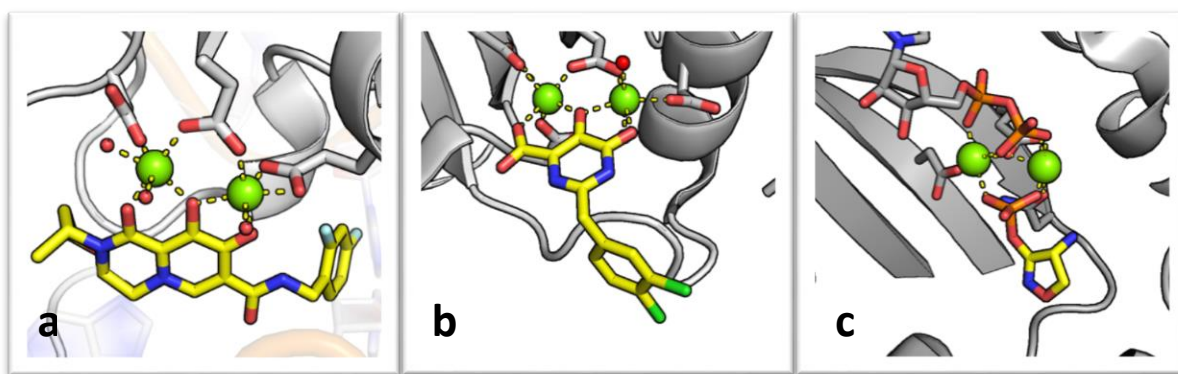
others if an obvious base is present in close proximity using available crystallographic structures [27,49,50]. The identity of such residues is not immediately obvious in some cases, and await more accurate active site geometries [55].

Another emerging possibility in specific systems, such as Ras, is to transfer the proton by an amide-imide tautomerization of e.g., a nearby glutamine (path **E**) [52,53]. This scenario requires an imide formation that is known to be highly endergonic, which itself does not rule the option out to stabilize even higher energy TSs. However, Grigorenko and co-workers [52,53] suggest a barrier of only *ca.* 4 kcal/mol for the nucleophilic substitution step, which is much lower than any other mechanistic alternatives.

## 5. Role of Magnesium Ions

Metal ions are essential in most phosphate catalytic reactions contributing to the fine-tuned pre-organization typical in enzyme catalysis [56]. Their roles are in not just coordinating ligands, but in altering the pKa of their coordinated groups [57,58]. In one-metal ion catalytic enzymes, the phosphate leaving group is always coordinated by the ion in the active conformation of the enzyme, thereby stabilizing the product. Many enzymes use the so-called two-metal ion catalytic mechanism [59], where a second metal ion coordinates the incoming nucleophile thereby activating them for easier deprotonation [60]. Additionally, two ions may also be present to activate the leaving group, such as for kinases, and occasionally even three ions are required for activity in some phosphate catalytic enzymes such as alkaline phosphatases [61,62], aminoacyl ligases [63] and nudix hydrolases [64,65]. Polarization can also be achieved with the help of positively charged active site residues. In ATPases and GTPases, an Arg residue, the so-called “Arginine finger” has an essential TS-stabilizing electrostatic effect, ensuring the correct catalytically active assembly by bringing the Arg residue from a separate subunit to contact the  $\gamma$ -phosphate [66]. Consequently, to correctly describe the enzyme mechanism, the metal ion with its coordination sphere must be modelled in the QM region together with all the active site residues contributing to the coupled phosphate bond breaking and formation as well as the proton transfer steps.

Magnesium ions are the most frequently occurring ions in the catalytic pockets of phosphate-related enzymes [51,67–69]. Accordingly, they also play a role in drug discovery to bind ligands directly (Figure 3). Less commonly,  $Mn^{2+}$  and  $Zn^{2+}$ , occasionally even  $Cu^{2+}$  or  $Co^{2+}$  can also act as the catalytic ion [65,70]. Importantly,  $Ca^{2+}$  ions typically inhibit phosphate catalytic enzymes, which also underlines the key role of  $Mg^{2+}$  as a Lewis acid. We have previously shown that  $Ca^{2+}$  has a lower polarization effect in its first coordination shell, which results in less polarization and decoupled proton transfer in phosphate cleavage mechanisms, leading to the loss of activity [71]. As  $Mg^{2+}$  and  $Ca^{2+}$  are the most abundant divalent metal ions in seawater, their specific roles in activating and inhibiting phosphate catalysis likely evolved to play key signalling biological roles by fine-tuning the enzyme catalytic activities [58]. This is supported by the inhibitory role of  $Ca^{2+}$  ions replacing  $Mg^{2+}$ , despite that they are structurally similar, underlining their key role as a Lewis acid. The reason behind that is that  $Ca^{2+}$  being a softer Lewis acid due mainly to its size, has a lower polarization effect in its first coordination shell [71].



**Figure 3.** Structural representation of three different inhibitors in complex with three important pharmaceutical phosphate catalytic enzyme targets: HIV-integrase **(a)**, RNase H **(b)** and DDL **(c)**. All ligands directly coordinate the active site Mg ions. PDB IDs used are **3OYA**, **3HYF** and **4C5A**, respectively.

Divalent metal ions have a slow exchange of their tightly-bound first-coordination shell ligands. This results in much longer timescales to identify the optimal coordination geometries, restricting simulations to local minima used at the initial starting geometries in simulations, including QM/MM but also MD studies. Remarkable efforts in sampling enhancement of metal coordination are on the way [72], but there is still room for developing generally applicable methods. Therefore, due to the lack of reliable experimental information regarding the precise active site structure in many cases, metal ion placement may render computational mechanistic studies inconclusive and awaiting further validation [31,32].

## 6. Transient Third Metal Ion

Polymerases have been long known as the prototype examples of two-metal ion catalytic enzymes. Intriguingly, recent experimental evidence from Gao and Yang on DNA polymerase  $\eta$  has demonstrated the possibility of a third metal ion transiently entering the active site during catalysis using time-resolved crystallography [73,74]. The same phenomenon was observed in DNA polymerase  $\beta$  [75], RNase H [76] and HIV RNase [77]. The question arises whether or not the role of these transient ions is essential, and if they are required for the catalytic step [78–81] or only to support product release [15,55,77,82]. Clarifying this role is particularly difficult due to the movements of the ions that occur on timescales inaccessible to unbiased QM/MM simulations. A current major challenge therefore remains: how to sample the slow catalytic steps discovering the precise reaction mechanism and the concurrently occurring conformational changes, including the transient conformational changes of ions and their coordinating ligands at the active site pockets.

## 7. Chemomechanical coupling

We can computationally decouple chemical steps from the conformational changes [83,84], which allows us to sample the latter using faster classical MD calculations. However, in some systems these events may not be independent, and thus this assumption of modelling molecular motions separately from the bond formation/cleavage might require further validation. Currently, tackling these complex conformational changes coupled to chemical activity are not routinely handled, but developments are underway. Particularly promising are novel machine learning based force fields that offer the speed of MD simulations, but with the possibility of bond formation/breakage, trained using accurate *ab initio* calculations [85,86].



## 8. Conclusions

Currently, QM/MM is the best approach to describe reactivity yet the complexity of the protein environment. Accurate QM DFT-based methods can reliably describe the reaction centres, including phosphates and metal ions with their coordination spheres. Molecular dynamics with a QM/MM level allow us to use enhanced sampling methods, which, if carefully designed, can be used to describe complex reaction mechanisms and the cooperativity of coupled reactions. If incorrect local minima were used as starting points often sampling is not sufficient to obtain catalytically competent structures computationally.

Additional challenges include the coupling of dynamical events with the slow chemical step. For example, it is currently still under debate, whether the transient role of a third  $\text{Mg}^{2+}$  ion in DNA/RNA polymerases is required for the catalytic step or for the subsequent product release. Even more challenging applications include RNA-based systems such as ribozymes and large protein/nucleic acid complexes. Novel approaches are also underway using machine learning for force fields coupled with enhanced sampling algorithms that might also be based on machine learning.

QM/MM methods also offer important applications. In drug discovery, structural roles of the metal ions can also play fundamental roles in the development of specific inhibitors. Traditional computational design tools were often unreliable to model the ligand interactions with metal ions, therefore ions were omitted in docking studies [87–89]. Nonetheless, there are several examples of successful drugs that interact strongly with active site-bound metal ions. Current inhibitors act on already different classes of targets, such as HIV integrase [90], RNase H [91], D-Ala-D-Ala Ligase [92], carbonic anhydrases [93], carboxypeptidase B [94] or adenosine deaminase [95] (Figure 3). However, there is still room for development of these types of inhibitors, as several major classes of drug targets currently lack such ligands, including kinases, polymerases, GTPases, ATPases, etc. QM/MM offers an accurate method to study these ion-bound ligands and to rationally design and engineer systems, transforming computations from a descriptive to a predictive tool.

### Conflict of interest

The authors declare no conflict of interest.

### Acknowledgement

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### Annotated Papers

- Special interest

- Outstanding interest

- [15] Yoon H, Warshel A: **Simulating the fidelity and the three Mg mechanism of pol  $\eta$  and clarifying the validity of transition state theory in enzyme catalysis.** *Proteins Struct Funct Bioinforma* 2017, **85**:1446–1453.

By performing empirical valence bond (EVB) calculations, the authors study the DNA polymerase  $\eta$  three metal ion mechanism and explain the fidelity of the polymerase while discussing the role of this third  $\text{Mg}^{2+}$ . Free energy profiles are obtained for the catalytic reaction with different base pairs and in presence and absence of the third  $\text{Mg}^{2+}$  suggesting its role in product stabilization rather than catalysis.

- [27] Lu X, Ovchinnikov V, Demapan D, Roston D, Cui Q: **Regulation and Plasticity of Catalysis in**

**Enzymes: Insights from Analysis of Mechanochemical Coupling in Myosin** *Biochemistry* 2017, **56**:1482–1497.

Studying different states of the myosin motor domain, the authors utilize advanced sampling techniques to evaluate the mechanism of ATP hydrolysis. The string calculations outline several possibilities for the proton transfer, favouring a base mediated two-step mechanism (path **D**).

•[49] Vithani N, Batra S, Prakash B, Nair NN: **Elucidating the GTP Hydrolysis Mechanism in FeoB: A Hydrophobic Amino-Acid Substituted GTPase**. *ACS Catal* 2017, **7**:902–906.

On the example of FeoB, this paper studies GTP hydrolysis using QM/MM metadynamics, exhibiting a good practice of mechanistic research. The work concludes that the base catalysed mechanism (path **D** in this review) is prominent involving a proton shuttle and removing the basic residues by mutagenesis, the two-water pathway (**A**) becomes the preferred mechanism.

•[66] Nagy GN, Suardíaz R, Lopata A, Ozohanics O, Vékey K, Brooks BR, Leveles I, Tóth J, Vértessy BG, Rosta E: **Structural Characterization of Arginine Fingers: Identification of an Arginine Finger for the Pyrophosphatase dUTPases**. *J Am Chem Soc* 2016, **138**:15035–15045.

This paper focuses on the structural characterization of an “Arginine Finger”, common among GTPases and ATPases, not seen with its essential function before in pyrophosphatases. Having a similar role as a  $Mg^{2+}$  ion, the electrostatic effect of this Arg enables the catalytically competent conformations of a P-loop and has a secondary role on catalysis for the dUTPases family. A structural comparison of this motif for NTP cleaving enzymes is also provided, pointing to an ancient structural origin.

••[77] Dürr SL, Bohuszewicz O, Suardiaz R, Jambrina PG, Peter C, Shao Y, Rosta E: **The Dual Role of Histidine as General Base and Recruiter of a Third Metal Ion in HIV-1 RNase H**. 2019, doi:10.26434/CHEMRXIV.8224538.V1.

In this work novel proton transfer pathways are proposed for RNase H enzyme reactions using the HIV-1 RNase H structures. Accurate DFT-based QM/MM calculations are coupled with Hamiltonian Replica Exchange and the string method. Conserved residues in the DEDDh motif are shown to play key roles, suggesting a general mechanism for this family of enzymes via the Glu as proton donor and the conserved histidine as a base for the proton transfer pathway. The His is also likely to play an important role in product release being involved with the third metal ion.

••[55] Stevens DR, Hammes-Schiffer S: **Exploring the Role of the Third Active Site Metal Ion in DNA Polymerase  $\eta$  with QM/MM Free Energy Simulations**. *J Am Chem Soc* 2018, **140**:8965–8969.

To obtain the free energy path of the system with three  $Mg^{2+}$  ions included in the active site, the authors utilized a finite temperature string method with a QM/MM treatment. They suggest that the third metal ion may play a secondary role, stabilizing the product through electrostatic interactions, while in a two-metal ion system supports a two-step mechanism, with the phosphate cleavage preceding the proton transfer.

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2. Saura P, Suardíaz R, Masgrau L, González-Lafont À, Rosta E, Lluch JM: **Understanding the Molecular Mechanism of the Ala-versus-Gly Concept Controlling the Product Specificity in Reactions Catalyzed by Lipoxygenases: A Combined Molecular Dynamics and QM/MM Study of Coral 8R-Lipoxygenase**. *ACS Catal* 2017, **7**:4854–4866.

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